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(54) Title: COMPOSITION AND METHOD FOR *IN VIVO* IMAGING OF AMYLOID DEPOSITS

(57) Abstract

An amyloid binding composition for *in vivo* imaging of amyloid deposits comprising a labeled amyloid protein or variant thereof which binds to amyloid deposits *in vivo*; and a pharmaceutically acceptable carrier, is described. Methods of detecting amyloid deposits and for diagnosing Alzheimer's Disease and Down's Syndrome are also described.

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COMPOSITION AND METHOD FOR IN VIVO IMAGING OF AMYLOID
DEPOSITS

Background of the Invention

The present invention relates to the identification of compositions which are suitable for use in *in vivo* imaging of amyloid deposits and methods related thereto. 10 More specifically, the present invention relates to a method of diagnosing Alzheimer's Disease.

15 Alzheimer's Disease ("AD") is the most common cause of dementia in the United States, and the presence of the disease is difficult to determine without invasive biopsies. The condition is characterized by impairments in memory, cognition, language and mobility, and these impairments progress over time.

Post-mortem slices of brain tissue from AD victims show that amyloid-containing senile plaques are a prominent feature of selective areas of the AD and the Down Syndrome brain. Divry, P., *J. Neurol. Psych.*, 27: 643-657 (1927); Wisniewski, et al., "Reexamination of the pathogenesis of the senile plaque," In Zimmerman, H.M. (ed.): *Progress in Neuropathology*, N.Y. (1973), Grune and Stratton, pp. 1-26. These plaques range in size from approximately 9 μm to 50 μm in diameter, when viewed by immunocytochemical methods designed to detect amyloid, and they vary in morphology and density. Majocha et al., *Proc. Natl. Acad. Sci. USA*, 85: 6182-6186 (1988). Classical staining methods can detect senile plaques as large as 200 μm . Tomlinson, et al., "Ageing and the dementias," In: Adams, J.H., et al., (ed.), *Greenfield's Neuropathology*, Edition 4, J. Wiley and Sons, N.Y., pp. 951-1006. These plaques are most often found in the cerebral cortex, but they also occur in deeper grey matter, including the amygdaloid nucleus, the corpus striatum, and the diencephalon. Plaques have also been described in the cerebellum. Pro, et al., *Neurology*, 30: 820-825 (1980). Senile plaques are composed of

extracellular amyloid, reactive cells, and degenerating neurites that contain Paired Helical Filaments, lysosomes, abnormal mitochondria and astrocytic processes. Wisniewski, et al., *supra* (1973). The 5 mechanisms responsible for the excessive accumulation of amyloid, the major proteinaceous component of senile plaques, have been recently addressed at the protein chemistry, molecular biology and genetic level.

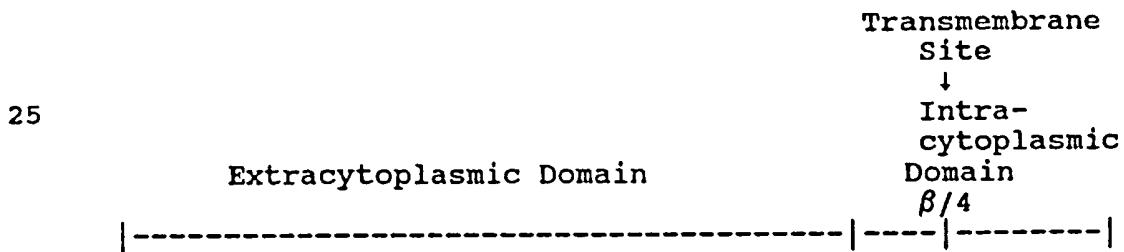
Specifically, amyloid is composed of fibrils of 10 4-8 nm in diameter that form the core of senile plaques. Mertz et al., *Acta Neuropathol.*, 60: 113-124 (1983). The amyloid is readily demonstrated by application of thioflavin S or Congo red to brain sections. In the latter case, polarized light causes amyloid to appear 15 with a characteristic yellow-green color. The staining property reflects the presence of twisted beta-pleated sheet fibrils, as noted above. A detailed discussion of the biochemistry and histochemistry of amyloid can be found in Glenner, *N. Engl. J. Med.*, 302: 1333-1343 20 (1980).

Vascular amyloidosis, referred to as congophilic angiopathy, has been recognized since the early part of this century as a significant aspect of the microscopic pathology of Alzheimer's Disease. Vinters, et al. 25 *Stroke*, 18: 311-324 (1987). Over 90% of Alzheimer cases have congophilic angiopathy. Glenner, et al., *Ann. Pathol.*, 1: 120-129 (1981). Similar to parenchymal amyloid deposits, vascular amyloid is demonstrated by characteristic thioflavin S and Congo red staining 30 reactions. The parieto-occipital cortex is usually more affected than that in the frontal and temporal lobes. Tomlinson et al., *supra* (1984).

In vascular amyloidosis, the amyloid appears to infiltrate the micro-vasculature, and affected vessels 35 often pass from the leptomeninges into the cortex. Small cerebral vessels with arterioles that appear as thickened

tubes are observed. The changes include the small pial and intracortical arterioles, the leptomeningeal vessels and the intracortical capillaries. Tomlinson et al., *supra* (1984). Immunocytochemical and electron microscopic studies have indicated that the amyloid component of senile plaques are often observed in close proximity to affected microvessels. Allsop, et al. *Neurosci. Lett.*, 68: 252-256 (1986). However, the angiopathy may occur without senile plaques. Montjoy, et al., *J. Neurol. Sci.*, 57: 89-103 (1982).

The principle component of both cerebral (senile plaques) and vascular amyloid is the 4.2 kilodalton peptide, β -amyloid, which is also referred to as β /A4 and A4. Glenner et al., *Biochem. Biophys. Res. Commun.*, 120: 885 (1984). β /A4 is derived from a parent molecule, the amyloid precursor protein (APP). Kang et al., *Nature*, 325: 733-736 (1987). At least three major variants of APP are known, having 695, 751 and 770 amino acids, respectively. In all three variants, the site of the β /A4 peptide is in the same relative 3'-end location, as follows:



Kang et al., *supra* (1987) showed through cloning APP-695 that APP has a large extracellular domain, a transmembrane domain (which gives rise to the β /A4 peptide) and an intracytoplasmic domain (See Figure 9). The signal sequence, for transport through the endoplasmic reticulum membrane, is followed by a region rich in cysteine, which suggests that disulfide bridges may stabilize this portion of the structure. Within the next 100 residues are a stretch of 7 uninterrupted

threonine residues and a region containing 28 glutamic acid residues and 17 aspartic residues. Marotta, et al. *J. Mol. Neurosci.*, 3: 111-125 (1992) suggest that this domain could bind cations extensively and may have physiological significance. Sodium dodecyl sulfate ("SDS") may be bound to a lesser extent than usual due to this domain. The region from residue 290 to 597, at which point the β /A4 site begins, contains two potential N-glycosylation sites at positions 467-469 and 496-498. The β /A4 peptide (residues 596-638 or 639) is either 42 or 43 amino acids in length and partly includes the putative transmembrane domain (amino acids 625-648). The C-terminal region of the APP is relatively small, consisting of 57 residues.

Following the transmembrane region, lysine residues are present (residues 649-651) which, according to Kang et al. *supra* (1987), could interact with phospholipid head groups in the membrane. This feature has been described for the junction between membrane and cytoplasmic domains of cell-surface receptors. One site (amino acids 684-686) is a potential glycosylation sequence.

Gandy et al. report that during in vitro studies of synthetic peptides corresponding to the cytoplasmic domain, it was observed that protein kinase C rapidly catalyzed the phosphorylation of a peptide corresponding to amino acid residues 645-661 on ser-655. Gandy et al., *Proc. Natl. Acad. Sci. USA*, 85(16): 5218-5221 (1988), suggesting that this site may be an important control region for amyloid metabolism and its interaction with other intracellular regulatory elements.

Recent research has also focused on the biological activity of β /A4. Specifically, it has been noted that this peptide and its fragments are trophic, toxic and or amnestic at various concentrations. Also, β /A4 forms insoluble aggregates (self-aggregates) under various

conditions, and the neurotoxicity of β /A4 is related to the aggregation process. Kirshner, et al., *Proc. Natl. Acad. Sci. USA*, 84: 6953-6957 (1987) and Maggio, *Annu. Rev. Neurosci.*, 11: 13-28 (1988). Maggio et al., also 5 studied the aggregation properties of radioiodinated synthetic β /A4 peptides *in vitro*. *Proc. Natl. Acad. Sci. USA*, 89: 5462-5466 (June 1992).

Pike et al., *J. Neurosci.*, 13(4): 1676-1687 (1993) 10 tested the aggregation properties of an overlapping series of synthetic β -amyloid peptides and compared them with their neurotoxic properties *in vitro*. They discovered that few peptides assembled into aggregates immediately after solubilization but that over time peptides containing the highly hydrophobic B29-35 region 15 formed stable aggregations. In short-term cultures, neurotoxicity was associated with those peptides demonstrating significant aggregations.

Thus far, diagnosis of AD has been achieved mostly 20 through clinical criteria evaluation, brain biopsies and post mortem tissue studies. However, recent work has focused on immunoassay methods for detecting markers of AD in body fluids such as spinal fluid and also in *in situ* hybridization studies using nucleic acid probes. World Patent No. 92/17152 by Potter; Warner, M., *Anal. Chem.*, 59: 1203A (1987); U.S. Patent No. 4,666,829 by 25 Glenner et al. In U.S. application no. 105,751, the contents of which is hereby incorporated by reference, Marotta et al. describe anti- β /A4 antibodies for purposes of *in vitro* and *in vivo* diagnostic methods.

Glenner et al., *supra*, teach the use of the β /A4 30 peptide, or fragments thereof, for the production of antibodies which recognize the antigenic determinants of the polypeptide or homologues thereof. Glenner et al. further teach the use of the disclosed polypeptide for 35 the production of nucleic acid probes which hybridize with the gene encoding the polypeptide. One such

Summary of the Invention

One object of the present invention is to provide an amyloid binding composition for *in vivo* imaging of amyloid deposits comprising a labeled amyloid protein 5 which binds to amyloid deposits *in vivo* and a pharmaceutically acceptable carrier.

Another object of the present invention is to provide an *in vivo* method for detecting amyloid deposits in a subject comprising the steps of administering to a 10 subject a detectable quantity of an amyloid binding composition comprising a labeled amyloid binding protein and a pharmaceutically acceptable carrier and detecting the binding of the labeled protein to the amyloid deposit.

15 Another object of the present invention is to provide a method of diagnosing an amyloidosis-associated disease, such as Alzheimer's Disease and Down Syndrome, by applying the above method to the detection of amyloid deposits in subjects suspected of having an amyloidosis- 20 associated disease.

The amyloid binding protein of the present invention includes all variants of the amyloid protein which bind to amyloid deposits *in vivo*.

Brief Description of the Drawings

25 Figure 1 shows a chart setting forth chemically documented amyloidosis with protein types.

Figure 2 depicts a PAGE-SDS gel of the A4-0 synthetic amyloid peptide. The amyloid polypeptide of 28 residues, corresponding to the previously reported sequence of 30 Masters et al., Proc. Natl. Acad. Sci. USA 82: 4245-4249 (1985) was synthesized on a Biosearch SAM2 synthesizer using the general procedure of Merrifield, J. Am. Chem. Soc., 85: 2149-2154 (1963). Purification was achieved with a 3 X 65 cm column of Sephadex G50 (10-40 μ). The 35 peptide (10 μ g) was suspended in sample buffer containing 2% SDS (Brown, et al., J. Neurochem., 40: 299-308 (1983))

and 9.5 M urea. Electrophoresis was carried out on a uniform 10% gel containing 0.1% SDS. (A) Molecular weight markers: phosphorylase B (94 kd), bovine serum albumin (68 kd), ovalbumin (43 kd), carbonic anhydrase (29 kd), trypsin inhibitor (22 kd), lysozyme (14.5 kd). (B) The synthetic amyloid peptide ran as a sharp band at the front of the gel and as an aggregated form of higher molecular weight.

Figure 3 depicts gel electrophoresis of the synthetic peptides A4-O and P2 (APP amino acids 413-429). (Panel a): Twenty ug of A4-O (lane 1) and P2 (APP amino acids 413-429) (lane 2) were analyzed by 18% SDS-PAGE (acrylamide : bisacrylamide = 30 : 0.8). (Panel b): Analysis of A4-O and P2 (APP amino acids 413-429) on 11% SDS/urea-PAGE (acrylamide : bisacrylamide = 20 : 1). Lanes 1-3 containing A4-O (10 ug) were incubated with 2% SDS and 5% 2-ME at 95°C for 5 minutes (Lane 1), 30 min (Lane 2) and 60 minutes (Lane 3). Lane 4 contained P2 (10ug). Gels were stained with Coomassie Brilliant blue R-250. Molecular weights are shown on the right (Kd).

Figure 4 shows slot blots of immunostained A4-O after addition of itself or a second A4 homologue. This assay depicts the increase staining intensity after A4 homologues are added to one another. This reflects the ability of homologues to self-aggregate and thus increase the staining intensity. In each case, the slot contained 1 ug of A4 peptide. To each, 2.5, or 10.0 ug of exogenous peptide was added, as indicated. The blots were then immunostained (see descriptions of Figures 5, 6 and 7).

Figure 5 shows immunoblots with and without exogenous A4-O peptide. Density values of the immunoreaction products of A4-O with and without exogenous peptides after reaction with 10H3. The values of the bars correspond to the density of blots shown in Figure 4. The description of Figure 4 indicates the condition of

each blot with regard to the exogenous peptide that was added to the blotted peptide prior to addition of 10H3. The height of the bars above the black bar (no peptide addition) is a measure of the extent to which the 5 exogenous peptide bound to the attached peptide on the filter paper and increased the density of immunostaining of the complex.

At the three indicated concentrations (2.5, 5.0 and 10.0 ug/ml) A4-0 was added to the A4-0 that was already 10 present at a concentration of 1 ug. The blot was then immunostained to develop the colored reaction product. The data were derived from scanning Figure 4.

15 Figure 6 shows immunoblots with and without exogenous A4-H peptide. At the three indicated concentrations (2.5, 5.0 and 10.0 ug/ml) A4-H was added to the A4-0 that was already present at a concentration of 1 ug. The blot was then immunostained to develop the colored reaction product. The data were derived from scanning Figure 4. See description of Figure 5 for more details.

20 Figure 7 shows immunoblots with and without exogenous Op1 peptide. At the three indicated concentrations (2.5, 5.0 and 10.0 ug/ml) Op1 was added to the A4-0 that was already present at a concentration of 1 ug. The blot was then immunostained to develop the colored reaction 25 product. The data were derived from scanning Figure 4. See description of Figure 5 for more details.

Figure 8 shows the reactivity of 10H3 towards A4-0 (upper panel).

30 Figure 9 (SEQ ID NOS:11 and 12) is the nucleotide sequence and predicted amino acid sequence of cDNA encoding the precursor protein (APP) of the β /A4 with the β /A4 region boxed, as set forth in Kang et al., *supra*, (1987).

Detailed Description of the Preferred Embodiments

Applicants have discovered that an amyloid binding composition comprising a labeled amyloid protein may be used *in vivo* for detecting the presence and 5 location of amyloid deposits. The amyloid binding composition of the present invention comprises a labeled amyloid protein and a pharmaceutically acceptable carrier. This protein is any natural or synthetic protein which binds to amyloid deposits *in vivo*. In one 10 embodiment, the protein is the β -amyloid polypeptide (β /A4 peptide), which in its longest form has 42 to 43 amino acid residues, as shown in Figure 9. See Masters, et al., *Proc. Nat. Acad. Sci., USA.*, 82: 4245-4249 (1985).

15 As noted above, β /A4 is derived from a larger amyloid precursor protein having from 695 to 770 amino acids. See Kang et al., *Nature*, 325: 733 (1987). The term "amyloid deposit" includes amorphous, eosinophilic materials that are associated with amyloidosis, a disease complex 20 including over 20 different clinically defined syndromes, as discussed above. Chemically, amyloid deposits are proteinaceous, and their chemical compositions are unique for each of the clinical syndromes with which they are associated, as set forth in Figure 1. Preferably, the 25 amyloid deposit of the present invention is that found in the brain of Alzheimer's Disease patients. As noted above, such amyloid deposits are found in senile plaques in selected areas of the AD brain and are composed of fibrils of 4-8 nm diameter. These plaques are detected 30 by application of thioflavin S or Congo red to brain sections and in the latter case, appear yellow-green under polarized light. They have twisted beta-pleated sheet fibrils and are further characterized by Glenner, *N. Eng. J. Med.*, 302: 1333-1343 (1980). In another 35 embodiment, the amyloid deposit of the present invention is that which is associated with vascular amyloidosis, as

described in Vinters, *Stroke*, 18: 311-324 (1987).
Vascular amyloid deposits infiltrate the cerebral micro-
vasculature. Similar to amyloid deposits found in
senile plaques in the parenchyma of the AD brain,
5 vascular amyloid deposits have characteristic thioflavin
S and Congo red staining reactions. Montjoy et al., *J.
Neurol. Sci.*, 57: 89-103 (1988).

The term "amyloidosis-associated disease" includes
any disease characterized by local or systemic amyloid
10 deposits. (See Figure 1) Preferably, the amyloidosis-
associated disease of the present invention is
Alzheimer's Disease or Down Syndrome.

In addition to amyloid protein purified from natural
sources such as cerebrovascular tissue, as described
15 hereinafter, amyloid protein of the present invention
includes recombinant and synthetic amyloid protein and
variants of the naturally occurring, recombinant and
synthetic protein. In a preferred embodiment, the
amyloid protein of the invention comprises the β -amyloid
20 polypeptide and variants thereof. The category of
"variants" includes, for example, a fragment of the β -
amyloid polypeptide or any homologous amino acid sequence
or amino acid addition, wherein the resulting polypeptide
has the same or similar function as the natural occurring
25 polypeptide in that it binds to amyloid deposits *in vivo*.
In one embodiment, the amyloid protein of the present
invention is comprised of the β -amyloid polypeptide or
variant thereof and amino acids from the APP protein
30 which are from regions of the APP protein which are
either adjacent or non-adjacent to the β -amyloid
polypeptide. For example, in one embodiment, the amyloid
protein of the present invention comprises:

(A) The β /A4 peptide alone (SEQ ID NO:2):
Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-
35 Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-

Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr; or

(B) The β /A4 peptide plus the amino acids of the transmembrane domain of the APP (SEQ ID NO:3):

5 Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Ile-Val-Ile-Thr-Leu-Val-Met-Leu; or

(C) The β /A4 peptide plus the remaining C-terminal 10 amino acids of the entire APP (SEQ ID NO:4):

Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Ile-Val-Ile-Thr-Leu-Val-Met-Leu-Lys-Lys-Gln-15 Tyr-Thr-Ser-Ile-His-His-Gly-Val-Val-Glu-Val-Asp-Ala-Ala-Val-Thr-Pro-Glu-Glu-Arg-His-Leu-Ser-Lys-Met-Gln-Gln-Asn-Gly-Tyr-Glu-Asn-Pro-Thr-Tyr-Lys-Phe-Phe-Glu-Gln-Met-Gln-Asn; or

(D) The β /A4 peptide with the preceding 10 amino 20 acids of the APP (SEQ ID NO:5):

Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Lys-Met-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr; or

(E) The β /A4 peptide with any other APP amino acids 25 attached to it that are not normally adjacent (SEQ ID NO:6):

X-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-30 Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr-Y,

wherein X and Y are one or more APP amino acids which are not adjacent to β /A4 in the nature; and

(F) any fragment of (A)-(E), wherein said fragment 35 is large enough to bind amyloid deposit *in vivo*.

The term "fragment" includes a linear amino acid subsequence of the β -amyloid polypeptide, wherein such fragment binds amyloid deposits *in vivo*. A variant which contains an amino acid sequence variation or substitution 5 is a homologous sequence. "Homology" between two sequences connotes a likeness short of identity indicative of a derivation of the first sequence from the second. For example, a polypeptide is "homologous" to β -amyloid polypeptide if it contains an amino acid sequence 10 similar enough to the natural sequence so as to confer the same or similar amyloid binding property as the natural β -amyloid polypeptide. Such a sequence may be only a few amino acids long and may be a single linear sequence or one or more linear sequences which confer 15 binding activity to the polypeptide when amino acids from separated portions of a linear sequence are spatially juxtaposed after protein folding. The variants encompassed by this invention can be ascertained, for example, by the *in vitro* quantitative assays describe 20 below in Examples 3-7. That is, applicants have conducted a series of studies involving the addition of increasing concentrations of β -amyloid polypeptide variants to a solid support containing a specific peptide called A4-0. The increase in density of immunostain 25 using an anti-A4-0 monoclonal antibody, 10H3, described in U.S. Patent application no. 105,751 by Marotta, et al., incorporated by reference above, was measured. Based upon this work, it was possible to determine which peptides were suitable for use in the *in vivo* methods, 30 according to the invention. Other poly- and/or monoclonal antibodies suitable for this assay can be produced by methods well known in the art. See Kennett et al., *Monoclonal Antibodies- Hybridomas: A New Dimension in Biological Analysis*, Plenum Press (1980) 35 Protein which qualifies as "amyloid protein" according to the above criteria can be produced by

methods known and emerging in the art, including conventional reverse genetic techniques, i.e., by designing a genetic sequence based upon an amino acid sequence or by conventional genetic splicing techniques.

5 For example, β -amyloid polypeptide variants can be produced by techniques which involve site-directed mutagenesis or oligonucleotide-directed mutagenesis. See, for example, "Mutagenesis of Cloned DNA," in CURRENT PROTOCOLS IN MOLECULAR BIOLOGY 8.0.3 et seq. (Ausubel, et 10 al. eds. 1989) ("Ausubel").

Other amyloid protein variants within the present invention are molecules that correspond to a portion of the β -amyloid polypeptide, but are not coincident with the natural molecule, and display the binding activity of 15 the natural molecule when presented alone or, alternatively, when linked to a carrier or biologically active signal sequence that permits proteins to pass through membranes. See von Heijne, G., *J. Mol. Biol.*, 184: 99-105 (1985). An amyloid protein variant of this 20 type could represent an actual fragment, as discussed above, or could be a polypeptide synthesized *de novo* or recombinantly.

To be used in recombinant expression of amyloid protein or amyloid protein variant, a polynucleotide 25 molecule encoding such a molecule would preferably comprise a nucleotide sequence, corresponding to the desired amino acid sequence, that is optimized for the host of choice in terms of codon usage, initiation of translation, and expression of commercially useful 30 amounts of, for instance, β -amyloid polypeptide or β -amyloid polypeptide variant. Also, the vector selected for transforming the chosen host organism with such a polynucleotide molecule should allow for efficient 35 maintenance and transcription of the sequence encoding the polypeptide. The encoding polynucleotide molecule may code for a chimeric protein; that is, it can have a

nucleotide sequence encoding a biologically active part of the β -amyloid molecule operably linked to a coding sequence for a non- β -amyloid moiety, such as a signal peptide for the host cell.

5 For instance, in order to isolate a DNA segment which encodes a β -amyloid molecule, total DNA from cerebrovascular tissue can be prepared according to published methods. See, for example, Maniatis, et al., MOLECULAR CLONING: A LABORATORY MANUAL (Cold Spring 10 Harbor Laboratories, NY 1982); Baess, *Acta Pathol. Microbiol. Scand. (Sect. B)*, 82: 78084 (1974). The DNA thus obtained can be partially digested with a restriction enzyme to provide an assortment of genomic fragments. An enzyme with a tetranucleotide recognition 15 site, such as *Sau3A* (*MboI*), is suitable for this purpose. The fragments from such a partial digestion then can be size-fractionated, for example, by sucrose gradient centrifugation (see Maniatis, *supra*) or by pulsed field gel electrophoresis (See Anad, *Trends in Genetics*, 20 November 1986, at pages 278-83), to provide fragments of a length commensurate with that of DNA encoding the β -amyloid molecule. Molecular cloning of amyloid cDNA derived from mRNA of the Alzheimer brain and the expression thereof is described in detail in Zain et al., 25 *Proc. Natl. Acad. Sci. USA.*, 85: 929-933 (1988) and Marotta et al., *Proc. Natl. Acad. Sci. USA.*, 86: 337-341 (1989), respectively, both of which are herein incorporated by reference.

According to well-known methods described, for 30 example, in Ausubel at 5.0.1 et seq., the selected fragments can be cloned into a suitable cloning vector. A DNA sequence thus obtained could be inserted, for example, at the *BamH1* site of the *pUC18* cloning vector which is transfected into appropriate host cells such as 35 *E. coli* or a mammalian cell. A variety of screening

mechanisms known in the art of the invention can then be used to identify clones containing the β -amyloid gene.

In another embodiment of the invention, amyloid protein of the present invention is purified from tissue samples. For example, brains from Alzheimer's Disease post-mortum patients are histologically sectioned and stained with Congo Red dye. Upon visualization with a polarizing microscope, amyloid deposits can be identified by their green color. Brains exhibiting extensive cerebrovascular amyloidosis are used as source for purified amyloid protein. After removal of contaminants from the amyloid containing vessels of the meninges, the meningeal tissues are homogenized and centrifuged to yield a brownish layer rich in amyloid fibrils. This layer is then digested with collagenase, solubilized in 6M guanidine HCl, pH 8.0 and centrifuged. The supernatant containing the solubilized protein is desalted by dialysis and gel exclusion column chromatography and high performance liquid chromatography is used to purify the polypeptide. The amino acids for the purified protein (e.g., β -amyloid polypeptide) are then sequentially cleaved in an automated amino acid sequencer, such as a Beckman 890 C spinning cup sequencer, and analyzed by high performance liquid chromatography in order to determine the amino acid sequence of the amyloid protein. See Glenner & Wong, *Biochem. Biophys. Chem. Res. Commun.*, 120: 885 (1984).

In another embodiment, amyloid protein and variants thereof can be produced in accordance with published methods. For instance, Kirschner et al., *Proc. Natl. Acad. Sci. USA*, 84: 6953-57 (1987) used an ABI Synthesizer model 380 B (Applied Biosystems, Foster City, CA) to synthesize synthetic β -amyloid peptides consisting of residues 1-28 and homologues thereof. General methods for peptide synthesis can be found in Clark Lewis et al., *Science*, 231: 134 (1986). See also, Hilbich et al., *J.*

Mol. Biol., 218: 149-163 (1991); Majocha et al., *Proc. Natl. Acad. Sci. USA*, 85: 6182-6186 (1988); and U.S. Patent application No. 105,751 by Marotta et al.

5 The term "in vivo imaging" refers to any method that permits the detection of a labeled amyloid protein which binds to amyloid deposits located in a subject's body. A "subject" is a mammal, preferably a human. Often, particularly when the composition and method of the invention is directed to the diagnosis of Alzheimer's 10 Disease or Down Syndrome, the subject will manifest clinical symptoms of the suspected amyloidosis. These clinical symptoms are well-known to the practitioner of this invention and include loss of memory, and other impairments described above.

15 The amyloid binding composition of the present invention must be of a "detectable quantity." A detectable quantity is that which is sufficient to enable detection of the site of amyloid deposit location when compared to a background signal. The dosage of the 20 amyloid binding composition will vary depending upon such considerations as age, condition, sex, extent of disease in the patient, counterindications, and other variables, to be adjusted by the individual physician. Dosage can vary from 25 0.01 mg/kg to 2,0000 mg/kg, preferably 0.1 mg/kg to 1,000 mg/kg.

30 In accordance with this invention, the amyloid protein may be labeled by any of several techniques known to the art. See, e.g., Wagner et al., *J. Nucl. Med.*, 20: 428 (1979); Sundberg et al., *J. Med. Chem.*, 17: 1340 (1974) and Saha et al., *J. Nucl. Med.*, 6: 542 (1976).

35 The label is chosen based upon the type of detection instrument employed. For instance, a chosen radionucleotide must have a type of decay which is detectable for a given type of instrument. Another

consideration relates to the half-life of the isotope. The half-life should be long enough so that it is still detectable at the time of maximum uptake by the target, but short enough so that the host does not sustain deleterious radiation. Preferably, the chosen label will lack a particulate emission, but will produce a large number of photons in a 140-200 keV range, which may be readily detected by, for instance, conventional gamma camera. Suitable radioisotopes for purposes of this invention include, gamma-emitters, position-emitters, x-ray emitters and fluorescence-emitters. These radioisotopes include Iodine-131, Iodine-123, Iodine-126, Iodine-133, Bromine-77, Indium-111, Indium-113m, Gallium-67, Gallium-68, Ruthenium-95, Ruthenium-97, Ruthenium-103, Ruthenium-105, Mercury-107, Mercury-203, Rhenium-99m, Rhenium-105, Rhenium 101, Tellurium-121m, Tellurium-122m, Tellurium-125m, Thulium-165, Thulium-167, Thulium-168, Technetium-99m and Fluorine-18. The preferred radiolabel is Technetium-99m. Suitable paramagnetic isotopes for use in Magnetic Resonance Imaging (MRI), according to this invention, include ¹⁵⁷Gd, ⁵⁵Mn, ¹⁶²Dy, ⁵²Cr, and ⁵⁶Fe.

Administration to the subject may be accomplished intraventricularly, intravenously, intraarterially, via the spinal fluid or the like. Administration may also be intradermal or intracavitory, depending upon the body site under examination. After a sufficient time has lapsed for the labeled amyloid protein to bind with amyloid deposits, for example 30 minutes to 48 hours, the area of the subject under diagnosis is examined by routine imaging techniques such as MRI, SPECT and planar scintillation imaging. The exact protocol will necessarily vary depending upon factors specific to the patient, as noted above, and depending upon the body site under examination, method of administration and type of label used; the determination of specific procedures

would be routine to the skilled artisan. The distribution of the bound radioactive isotope and its decrease with time is then monitored and recorded. By comparing the results with data obtained from studies of 5 clinically normal individuals, the presence and location of amyloid deposits can be determined.

Thus, in one embodiment, the methods of the present invention is used to diagnoses an amyloidosis-associated disease. Where the site of examination is the brain, the 10 *in vivo* detection of amyloid deposits according to the methods of the present invention signifies a diagnosis of Alzheimer's Disease. The detection of amyloid deposits in the brain of patients manifesting clinical symptoms of Down Syndrome, signifies a diagnosis of Down Syndrome. 15 In that regard, applicants note that the gene for APP, located on chromosome 21, is over-represented in Down Syndrome individuals (Serra et al., Amer. J. Med. Gen. Supp., 7: 11-19 (1990)). Accumulations of amyloid occur in young Down Syndrome patients, with nearly 90% of Down 20 Syndrome subjects aged less than 30 years showing amyloid accumulation (Hyman, Prog. Clin. Biol. Res. 379: 123-142 (1992)). The Down Syndrome patient displays amyloid accumulations early in life, often by late teenage years. As adults, nearly 100% will develop Alzheimer Disease 25 (Cork, Amer. J. Med. Gen. Supp., 7: 282-539 (1990)). The neuropathology of Down Syndrome is essentially identical to that of Alzheimer Disease and includes β /A4 amyloid deposits in senile plaques. The Alzheimer - like lesions represent a major neuropathologic trait of the brain of 30 the Down Syndrome patient (Serra et al., Supra (1990)).

The amyloid-binding compositions of the present invention are advantageously administered in the form of injectable compositions. A typical composition for such purpose comprises a pharmaceutically acceptable carrier. 35 For instance, the composition may contain about 10 mg of

human serum albumin and from about 20 to 200 micrograms of the labeled amyloid protein per milliliter of phosphate buffer containing NaCl. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the like, as described in REMINGTON'S PHARMACEUTICAL SCIENCES, 15th Ed. Easton: Mack Publishing Co. pp 1405-1412 and 1461-1487 (1975) and THE NATIONAL FORMULARY XIV., 14th Ed. Washington: American Pharmaceutical Association (1975), the contents of which are hereby incorporated by reference. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil and injectable organic esters such as ethyloleate. Aqueous carriers include water, alcoholic/aqueous solutions, saline solutions, parenteral vehicles such as sodium chloride, Ringer's dextrose, etc. Intravenous vehicles include fluid and nutrient replenishers. Preservatives include antimicrobials, anti-oxidants, chelating agents and inert gases. The pH and exact concentration of the various components of the binding composition are adjusted according to routine skills in the art. See GOODMAN AND GILMAN'S THE PHARMACOLOGICAL BASIS FOR THERAPEUTICS (7th ed.). The skilled artisan would also readily appreciate that a suitable excipient or carrier would need to prevent aggregation of the binding composition prior to contacting the target amyloid deposit *in vivo*.

Particularly preferred amyloid binding compositions of the present invention are those that, in addition to binding to amyloid deposits *in vivo*, are also non-toxic at appropriate dosage levels, have a satisfactory duration of effect, and display an adequate ability to cross the blood-brain barrier. In this regard, United States Patent No. 4,540,564 discloses an approach for enhancing blood-brain barrier-penetrating ability by attaching a centrally acting drug species to a reduced,

biooxidizable, lipoidal form of dihydropyridine pyridinium salt redox carrier. Thus, in one embodiment, the composition of the present invention includes such a blood-brain barrier crossing enhancer carrier.

5 *In vivo* animal testing provides yet a further basis for determining dosage ranges, efficacy of transfer through the blood barrier and binding ability. Particularly preferred for this purpose is the "senile animal" model for cerebral amyloidosis -- animals such as
10 aged dogs or monkeys, which are known to develop variable numbers of Alzheimer-type cerebral senile plaques, see Wisniewski, et al., *J. Neuropathol. & Exp. Neurol.*, 32: 566 (1973), Selkoe, et al., *Science*, 235: 873 (1987) are tested for binding and detection efficacy. This *in vivo*
15 assay requires control-biopsy monitoring to confirm and quantify the presence of amyloid deposits.

Also, cellular models of amyloidosis have been prepared that overproduce β -amyloid polypeptide in animals for purposes of testing the efficacy of the amyloid binding compositions and methods of the present invention. See Marotta, et al. *Proc. Natl. Acad. Sci. USA*, 86: 337-341 (1989). Such cell models have been adapted to a behavior paradigm. See Tate-Ostroff, *Proc. Natl. Acad. Sci. USA* 89: 7090-7094, (1992). That is,
20 because AD patients suffer circadian rhythm dysfunction, this behavioral deficit was modeled in rats by a cell grafting techniques. PC12 cells transfected with the β -amyloid polypeptide C-terminal region of the APP were implanted into the suprachiasmatic nuclei ("SCN") of rats; the SCN is a primary circadian oscillator in mammals. Animals receiving amyloidotic cell grafts, but not animals receiving control cell grafts, exhibited
25 disrupted activity rhythms, although temperature rhythms were unaffected. The specificity of the disruption was similar to circadian dysfunction seen in AD patients.
30 The data supported an association between a defined
35

behavioral disruption and amyloid overexpression either directly or through the release of cellular factors as a consequence of amyloid overproduction.

Other suitable animal models for use in testing the 5 compositions and methods of the present invention are produced transgenically. For instance, Quon et al., *Nature*, 352: 239-241 (1991) used rat neural-specific enolase promoter inhibitor domain to prepare transgenic mice. See also, Wirak et al., *Science*, 253: 323-325 10 (1991). Still other models have been produced by Intracranial administration of the β /A4 peptide directly to animals (Tate et al., *Bull. Clin. Neurosci.*, 56: 131-139 (1991)).

Without further elaboration, it is believed that one 15 skilled in the art can, using the preceding description, utilize the present invention to its fullest extent. The following examples are merely illustrative and not limitative of the remainder of the disclosure in any way whatsoever.

EXAMPLES

As noted above, A4 is intended to be the same as β /A4, throughout the examples. The peptides used in the following Examples have the following structures:

5 **A4-O (peptides 1-28), SEQ ID NO:7:**

N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Ser-Ala-COOH

10 The A4-O(1-28) polypeptide that was reported in Masters, et al. *Proc. Nat'l. Acad. Sci. U.S.A.*, 82: 4245-4249 (1985) is the first 28 amino acids of the 4.2 Kd peptide derived from senile plaque cores of an AD brain. Masters, et al. have also shown that the naturally occurring peptide aggregates even in denaturing gels.

15 The A4-O(1-28) sequence of this invention was synthesized by Biosearch in San Rafael, CA. The underlined amino acids differ from A4-P(1-28), as shown below.

A4-H (peptides 1-28):

20 The A4-H peptide is the same as A4-O(1-28) except that it was synthesized by the Harvard Microchemistry Laboratory.

A4-P (peptides 1-28), SEQ ID NO:8:

25 N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Gln-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-COOH

30 This sequence was reported by Glenner and Wong, *supra*, (1984) and derived from vascular amyloid of the AD brain and from a Down Syndrome brain. Three of 28 amino acids are different from the A4-O/A4-H peptides (underlined).

A4-B (peptides 1-28), SEQ ID NO:9:

N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-COOH

35 This sequence was obtained from Bachem and is the 28 amino acid structure that is commonly determined from

5 molecular cloning studies (Kang, et al., *Nature (London)* 325: 733-736 (1987)). Unlike the Glenner and Wong, *supra*, sequence (A4-P(1-28)), it has Glu, not Gln, at position 11. And, unlike A4-O/A4-H, it has Asn-Lys and not Ser-Ala at the C-terminus.

10 Op1 (peptides 1-10), SEQ ID NO:10:
N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-COOH
A4(1-10) consists of the first 10 amino acids of the amyloid peptide derived from any source and is described in U.S. Patent No. 4,666,829 by Glenner et al. Thus far, this sequence appears conserved in all reports on amyloid that is derived from non-Familial AD cases. The A4-(1-10) antigen used in the present studies was synthesized by the Harvard Microchemistry Laboratory.

15 Summary of sequence variations: dashed line indicates sequence conservation among the peptides shown.

20 A4-O (1-28): N-----Glu-----Ser-Ala- | (Masters)
A4-H (1-28): N-----Glu-----Ser-Ala- | (Masters)
A4-P (1-28): N-----Gln-----Asn-Lys- | (Glenner)
A4-B (1-28): N-----Glu-----Asn-Lys- | (Kang)
Op1 : N-----|

Example 1. Self-aggregation of the A4-0 amyloid peptide in SDS/urea acrylamide gels

The synthetic β -amyloid 28-mer polypeptide, A4-0 (Masters, et al., *supra*) was analyzed by polyacrylamide gel electrophoresis (PAGE) procedures (Brown, et al., *J. Neurochem.*, 40: 299-308 (1983)) and was noted to have unusual aggregation properties. The peptide was dissolved in a PAGE sample buffer containing SDS and urea and was electrophoresed on a 10% gel containing SDS (See 5 description of Figure 2). After staining with Coomassie blue, the peptide appeared as a broad band at approximately 23-25 kd and a narrow band that migrated at the gel front during electrophoresis (See Figure 2). The higher molecular weight species appeared to be an 10 aggregate since it was eliminated by adding urea to the separating gel and, subsequently, a 3-4 kd band was 15 obtained (not shown). Polyclonal antiserum to the 28-mer was prepared and applied to nitrocellulose blots of an overloaded gel. The latter contained a series of 20 aggregated peptides of various apparent molecular weights, all of which reacted with the antiserum. Thus, the synthetic 28-mer had aggregational properties not unlike the naturally occurring A4-0 amyloid protein of 4 kd (Masters, et al., *supra*).

25 Applicants' studies demonstrating the aggregation properties of the A4-0 peptide were previously reported (Salim, et al., "Molecular Cloning of Amyloid cDNA from Alzheimer Brain Messenger RNA" in *Familial Alzheimer's Disease*, J.P. Blass et al. eds., Marcel Dekker, NY pp 30 153-165 (1988)).

Based upon the results shown in Figure 2, applicants concluded that even in the presence of strong denaturing agents and after electrophoresis, A4-0 strongly bound to 35 itself.

Example 2: Self-aggregation of A4-O peptide on highly cross-linked SDS/urea acrylamide gels

Applicants obtained confirmatory data using the highly cross-linked acrylamide gel system described by 5 Honda and Marotta, *Neurochem. Res.*, 17: 367-374 (1992).

When analyzed by this gel system containing SDS and urea, the synthetic peptide A4-O migrated as a broad series of bands below an apparent molecular weight of 10 15kDa (data not shown). However, when 6M urea was added to the PAGE system the peptide appeared as a sharp single 15 band of 15kDa (Figure 3) and smaller size bands were not observed even after silver staining (data not shown). By contrast, peptide P2(413-429), used as a control and corresponding to an extracytoplasmic region of the β /A4 precursor protein, migrated with the bromphenol blue dye front on both SDS-PAGE and SDS/urea-PAGE systems (Figure 3, lane 4). Since the theoretical molecular weight of the 28 amino acid peptide A4-O is 3,178 Da the results indicate that the band of 15kDa is an aggregate. 20 Migration of A4-O peptide bands on both gel systems was not affected by 2-ME nor by pre-treatment with 80% formic acid (data not shown).

The 15kDa was visible after peptide A4-O were treated for 5 minutes at 95°C prior to electrophoresis (Figure 3, 25 lane 1). When boiling time was increased to 30 minutes or 60 minutes the aggregate partly dissociated to a smaller size (Figure 3, lanes 2 and 3). This dissociation was not dependent on the presence of SDS and 2-ME in the sample buffer but rather on the time of heat 30 denaturation. These data were previously reported (Honda and Marotta, *supra*).

Applicants concluded that Figure 3 confirms that even 35 in the presence of strong denaturing agents and heat treatment after electrophoresis, A4-O strongly bound to itself.

Example 3: Self-aggregation of A4 peptides on immunoblots

5 Due to the desirability of obtaining a quantitative assay for the selection of β -amyloid polypeptides for the composition and methods of the present invention, applicants elected to use quantitative slot blots to test aggregation of peptides rather than tissue slices. The general immunoblotting procedure utilizing A4 peptides attached to a solid support and detectable by applied 10 anti-amyloid antibodies was reported earlier (Majocha, et al., *supra*, (1988)). In all cases, the monoclonal antibody used to detect A4 aggregates was 10H3 (2 ug/ml).

15 One microgram of each of the indicated peptides were added overnight at room temperature to Millipore P filter paper to which A4-O was attached. The peptides were dissolved in ICC buffer: 2% BSA, 0.3M NaCl, 20mM Tris, 0.01% Triton. The blots were immunoprocessed (Majocha, et al. *supra*.) and then optically scanned for density; the areas under the curves were integrated by means of an 20 LKB Laser Densitometer.

25 Peptides A4-O, A4-H and Op1 were applied to filters to which was bound peptide A4-O, the antigen used to prepare mab 10H3. The experiment was designed to test the competence of each of the applied peptides to bind to the bound peptide. While A4-O and A4-H have the same primary structure, it has been noted that peptides with identical sequences that are obtained from different sources may have non-identical properties. (See Figure 4).

30 The density of staining (the optical density of the immunoreaction product) is quantitated in Figures 5, 6 and 7. The OD is a measure of the extent of the aggregation since it will be related to the antibody concentration and thus the color reaction.

35 The density values shown in Figure 5 were obtained by densitometric scanning of the reaction product on

blots from which the control value (no primary antibody) was subtracted. A further control was one in which the mab 10H3 was added to blots containing Opl in the absence of added exogenous peptide. This control value 5 represents the antibody-antigen reaction without interference from added peptides.

Based upon the results presented in Figure 5, applicants concluded that A4-O bound to itself with an optimal concentration of 5.0 ug/ml.

10 **Example 4: Self-aggregation of A4-H peptides on immunoblots**

15 The experiment of Example 3 was repeated except that the exogenous peptide was A4-H. The data are shown in Figure 6 and based upon these results, applicants concluded that A4-H bound to A4-O at an optimal concentration of 2.5 ug/ml.

Example 5: Self-aggregation of Op1 peptides on immunoblots

The experiment of Example 3 was repeated except that the exogenous peptide was Op1. The data are shown in 5 Figure 7 and based upon these results, applicants concluded that Op1 bound to A4-O at an optimal concentration of 2.5 ug/ml.

Based upon the results presented in Figures 5, 6 and 10 7, applicants concluded that three peptides bound the filter-bound A4-O peptide and increased the extent to which 10H3 reacted. The reaction is concentration-dependent. The three peptides, A4-O, A4-H and Op1, aggregated to the attached A4-O. The Op1 10-mer reacted nearly as well or better, at 2.5 ug, as the larger 28- 15 mers.

Example 6: Specificity of 10H3 for both A4-O and Op1

The results shown in Figure 7 indicate that a small peptide, a ten-mer, was able to bind at least as well as 1-28-mers to an A4 substrate.

20 Thus, this assay, which measures the optical density of the reaction product between the added 10H3 mab and the Op1 peptide on the solid surface, reflected the presence of the exogenous peptide, as applicants previously demonstrated for the reaction between 10H3 and 25 A4-O.

With respect to Op1, additional studies were carried out to confirm the reactivity of 10H3. On separate solid supports (Millipore P paper) either the A4-O antigen 30 (2ug/slot) or the Op1 antigen (2 ug/slot) were absorbed using a slot blot apparatus. The results are shown in Figure 8, as follows:

Reactivity of 10H3 towards A4-O (upper panel):

Blot no.:

35 1. Immunostain lacking the primary antibody (10H3) showed no reactivity with the blot, as expected.

-30-

2. 10H3 was very strongly reactive with its own antigen, A4-O.
3. Soluble A4-O antigen added to the mix caused inhibition of 10H3 towards A4-O.
- 5 4. Soluble Opi added to the mix caused inhibition of 10H3 towards A4-O.
5. 10H3 was reactive towards the Opi antigen.
6. Soluble Opi added to the mix showed inhibition of 10H3 towards Opi.

10 The slot blots were quantified by densitometry and numerical values were obtained that indicated the extent of the reaction between 10H3 and antigens. These values are given below in Table I in which each numbered item refers to the blot number in Figure 8 and the description

15 given above:

Table I: Optical Density of Reaction Between 10H3 and Either A4-O or Opi Antigens in Blots of Figure 7

Slot Number:	1	2	3	4	5	6
OD Units:	0.03	0.70	0.31	0.18	0.16	0.07

Based upon the results presented in Figure 8 and Table I, applicants concluded that 10H3 is reactive with its own A4-O antigen as well as with the Opi peptide.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: THE MIRIAM HOSPITAL
(ii) TITLE OF INVENTION: Composition and Method for in Vivo Imaging of Amyloid Deposits

(iii) NUMBER OF SEQUENCES: 13

(iv) CORRESPONDENCE ADDRESS:
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(C) CITY: Washington, D.C.
(E) COUNTRY: USA
(F) ZIP: 20007-5109

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER:
(B) FILING DATE: 27 May 1994
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Gln Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
20 25

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15
Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30
Gly Leu Met Val Gly Gly Val Val Ile Ala Thr
35 40

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 52 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15
Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30
Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr
35 40 45
Leu Val Met Leu
50

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 99 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15
Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
20 25 30
Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr
35 40 45
Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val
50 55 60
Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys
65 70 75 80
Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln
85 90 95
Met Gln Asn

(2) INFORMATION FOR SEQ ID NO:5:

-33-

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 53 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe Arg His
1 5 10 15

Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe Ala Glu
20 25 30

Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val Gly Gly
35 40 45

Val Val Ile Ala Thr
50

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 45 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 1
- (D) OTHER INFORMATION: /note= "Xaa at position 1 corresponds to 1 or more APP amino acids which are not adjacent to B/A4 in nature."

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION: 45
- (D) OTHER INFORMATION: /note= "Xaa at position 45 corresponds to 1 or more APP amino acids which are not adjacent to B/A4 in nature."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Xaa Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln
1 5 10 15

Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile
20 25 30

Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Xaa
35 40 45

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: A4-O

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Ser Ala
20 25

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: A4-P

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Gln Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
20 25

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 28 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: A4-B

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
20 25

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

- (B) CLONE: Opl

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:11:

-35-

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3353 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 147..2234

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AGTTTCCTCG	GCAGCGGTAG	GCGAGAGCAC	GCGGAGGAGC	GTGCGCGGGG	CCCCGGGAGA	60
CGGCGGCGGT	GGCGGCGCGG	GCAGAGCAAG	GACGCCGGCGG	ATCCCACTCG	CACAGCAGCG	120
CACTCCGTGC	CCCGCGCAGG	GTCGCG	ATG CTG CCC	GGT TTG GCA	CTG CTC CTG	173
			Met Leu Pro	Gly Leu Ala	Leu Leu Leu	
			1	5		
CTG GCC	GCC TGG	ACG GCT	CGG GCG	CTG GAG	GTA CCC ACT	60
Leu Ala	Ala Trp	Thr Ala	Arg Ala	Leu Glu	Val Pro	221
10	15	20	25			
GCT GGC	CTG CTG	GCT GAA	CCC CAG	ATT GCC	ATG TTC TGT	269
Ala Gly	Leu Ala	Glu Pro	Gln Ile	Ala Met	Phe Cys Gly Arg	
30	35	40				
AAC ATG	CAC ATG	AAT GTC	CAG AAT	GGG AAG	TGG GAT	317
Asn Met	His Met	Asn Val	Gln Asn	Gly Lys	Trp Asp Ser Asp	
45	50	55				
GGG ACC	AAA ACC	TGC ATT	GAT ACC	AAG GAA	GGC ATC	365
Gly Thr	Lys Thr	Cys Ile	Asp Thr	Lys Glu	Gly Ile Leu Gln	
60	65	70				
CAA GAA	GTC TAC	CCT GAA	CTG CAG	ATC ACC	AAT GTG	413
Gln Glu	Val Tyr	Pro Glu	Leu Gln	Ile Thr	Asn Val Val Glu	
75	80	85				
CAA CCA	GTG ACC	ATC CAG	AAC TGG	TGC AAG	CGG GGC	461
Gln Pro	Val Thr	Ile Gln	Asn Trp	Cys Lys	Arg Gly Arg Lys	
90	95	100				
AAG ACC	CAT CCC	CAC TTT	GTG ATT	CCC TAC	CGC TGC	509
Lys Thr	His Pro	His Phe	Val Ile	Pro Tyr	Arg Cys Leu Val	
110	115	120				
TTT GTA	ACT GAT	GCC CTT	CTC GTT	CCT GAC	AAG TGC	557
Phe Val	Ser Asp	Ala Leu	Leu Val	Pro Asp	Lys Cys	
125	130	135				
CAG GAG	AGG ATG	GAT GTT	TGC GAA	ACT CAT	CTT CAC	605
Gln Glu	Arg Met	Asp Val	Cys Glu	Thr His	Leu His	
140	145	150				
GCC AAA	GAG ACA	TGC AGT	GAG AAG	AGT ACC	AAC TTG	653
Ala Lys	Glu Thr	Cys Ser	Glu Lys	Ser Thr	Asn Leu	
155	160	165				
ATG TTG	CTG CCC	TGC GGA	ATT GAC	AAG TTC	CGA GGG	701
Met Leu	Leu Pro	Cys Gly	Ile Asp	Lys Phe	Arg Gly	
170	175	180				
TGT TGC	CCA CTG	GCT GAA	GAA AGT	GAC AAT	GTG GAT	749

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Cys	Cys	Pro	Leu	Ala	Glu	Glu	Ser	Asp	Asn	Val	Asp	Ser	Ala	Asp	Ala	
190									195					200		
GAG	GAG	GAT	GAC	TCG	GAT	GTC	TGG	TGG	GGC	GGA	GCA	GAC	ACA	GAC	TAT	797
Glu	Glu	Asp	Asp	Ser	Asp	Val	Trp	Trp	Gly	Gly	Ala	Asp	Thr	Asp	Tyr	
205								210					215			
GCA	GAT	GGG	AGT	GAA	GAC	AAA	GTA	GTA	GAA	GTA	GCA	GAG	GAG	GAA	GAA	845
Ala	Asp	Gly	Ser	Glu	Asp	Lys	Val	Val	Glu	Val	Ala	Glu	Glu	Glu	Glu	
220						225				230						
GTG	GCT	GAG	GTG	GAA	GAA	GAA	GCC	GAT	GAT	GAC	GAG	GAC	GAT	GAG		893
Val	Ala	Glu	Val	Glu	Glu	Glu	Glu	Ala	Asp	Asp	Asp	Glu	Asp	Asp	Glu	
235					240				245							
GAT	GGT	GAT	GAG	GTA	GAG	GAA	GAG	GCT	GAG	GAA	CCC	TAC	GAA	GAA	GCC	941
Asp	Gly	Asp	Glu	Val	Glu	Glu	Glu	Glu	Ala	Glu	Glu	Pro	Tyr	Glu	Glu	Ala
250					255				260			265				
ACA	GAG	AGA	ACC	ACC	AGC	ATT	GCC	ACC	ACC	ACC	ACC	ACC	ACA	GAG		989
Thr	Glu	Arg	Thr	Thr	Ser	Ile	Ala	Thr	Thr	Thr	Thr	Thr	Thr	Glu		
270					275				280							
TCT	GTG	GAA	GAG	GTG	GTT	CGA	GTT	CCT	ACA	ACA	GCA	GCC	AGT	ACC	CCT	1037
Ser	Val	Glu	Val	Val	Arg	Val	Pro	Thr	Thr	Ala	Ala	Ser	Thr	Pro		
285					290				295							
GAT	GCC	GTT	GAC	AAG	TAT	CTC	GAG	ACA	CCT	GGG	GAT	GAG	AAT	GAA	CAT	1085
Asp	Ala	Val	Asp	Lys	Tyr	Leu	Glu	Thr	Pro	Gly	Asp	Glu	Asn	Glu	His	
300					305				310							
GCC	CAT	TTC	CAG	AAA	GCC	AAA	GAG	AGG	CTT	GAG	GCC	AAG	CAC	CGA	GAG	1133
Ala	His	Phe	Gln	Lys	Ala	Lys	Glu	Arg	Leu	Glu	Ala	Lys	His	Arg	Glu	
315					320				325							
AGA	ATG	TCC	CAG	GTC	ATG	AGA	GAA	TGG	GAA	GAG	GCA	GAA	CGT	CAA	GCA	1181
Arg	Met	Ser	Gln	Val	Met	Arg	Glu	Trp	Glu	Glu	Ala	Glu	Arg	Gln	Ala	
330					335				340			345				
AAG	AAC	TTG	CCT	AAA	GCT	GAT	AAG	AAG	GCA	GTT	ATC	CAG	CAT	TTC	CAG	1229
Lys	Asn	Leu	Pro	Lys	Ala	Asp	Lys	Lys	Ala	Val	Ile	Gln	His	Phe	Gln	
350					355				360							
GAG	AAA	GTG	GAA	TCT	TTG	GAA	CAG	GAA	GCA	GCC	AAC	GAG	AGA	CAG	CAG	1277
Glu	Lys	Val	Glu	Ser	Leu	Glu	Gln	Glu	Ala	Ala	Asn	Glu	Arg	Gln	Gln	
365					370				375							
CTG	GTG	GAG	ACA	CAC	ATG	GCC	AGA	CTG	GAA	GCC	ATG	CTC	AAT	GAC	CGC	1325
Leu	Val	Glu	Thr	His	Met	Ala	Arg	Val	Glu	Ala	Met	Leu	Asn	Asp	Arg	
380					385				390							
CGC	CGC	CTG	GCC	CTG	GAG	AAC	TAC	ATC	ACC	GCT	CTG	CAG	GCT	GTT	CCT	1373
Arg	Arg	Leu	Ala	Leu	Glu	Asn	Tyr	Ile	Thr	Ala	Leu	Gln	Ala	Val	Pro	
395					400				405							
CCT	CGG	CCT	CGT	CAC	GTG	TTC	AAT	ATG	CTA	AAG	AAG	TAT	GTC	CGC	GCA	1421
Pro	Arg	Pro	Arg	His	Val	Phe	Asn	Met	Leu	Lys	Lys	Tyr	Val	Arg	Ala	
410					415				420			425				
GAA	CAG	AAG	GAC	AGA	CAG	CAC	ACC	CTA	AAG	CAT	TTC	GAG	CAT	GTC	CGC	1469
Glu	Gln	Lys	Asp	Arg	Gln	His	Thr	Leu	Lys	His	Phe	Glu	His	Val	Arg	
430					435				440							
ATG	GTG	GAT	CCC	AAG	AAA	GCC	GCT	CAG	ATC	CGG	TCC	CAG	GTT	ATG	ACA	1517
Met	Val	Asp	Pro	Lys	Lys	Ala	Ala	Gln	Ile	Arg	Ser	Gln	Val	Met	Thr	
445					450				455							

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CAC CTC CGT GTG ATT TAT GAG CGC ATG AAT CAG TCT CTC TCC CTG CTC His Leu Arg Val Ile Tyr Glu Arg Met Asn Gln Ser Leu Ser Leu Leu 460 465 470	1565
TAC AAC GTG CCT GCA GTG GCC GAG GAG ATT CAG GAT GAA GTT GAT GAG Tyr Asn Val Pro Ala Val Ala Glu Glu Ile Gln Asp Glu Val Asp Glu 475 480 485	1613
CTG CTT CAG AAA GAG CAA AAC TAT TCA GAT GAC GTC TTG CCC AAC ATG Leu Leu Gln Lys Glu Gln Asn Tyr Ser Asp Asp Val Leu Ala Asn Met 490 495 500 505	1661
ATT AGT GAA CCA AGG ATC AGT TAC GGA AAC GAT GCT CTC ATG CCA TCT Ile Ser Glu Pro Arg Ile Ser Tyr Gly Asn Asp Ala Leu Met Pro Ser 510 515 520	1709
TTG ACC GAA ACG AAA ACC ACC GTG GAG CTC CTT CCC GTG AAT GGA GAG Leu Thr Glu Thr Lys Thr Val Glu Leu Leu Pro Val Asn Gly Glu 525 530 535	1757
TTC AGC CTG GAC GAT CTC CAG CCG TGG CAT TCT TTT GGG GCT GAC TCT Phe Ser Leu Asp Asp Leu Gln Pro Trp His Ser Phe Gly Ala Asp Ser 540 545 550	1805
GTG CCA GCC AAC ACA GAA AAC GAA GTT GAG CCT GTT GAT GCC CGC CCT Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val Asp Ala Arg Pro 555 560 565	1853
GCT GCC GAC CGA GGA CTG ACC ACT CGA CCA GGT TCT GGG TTG ACA AAT Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser Gly Leu Thr Asn 570 575 580 585	1901
ATC AAG ACG GAG GAG ATC TCT GAA GTG AAG ATG GAT GCA GAA TTC CGA Ile Lys Thr Glu Ile Ser Glu Val Lys Met Asp Ala Glu Phe Arg 590 595 600	1949
CAT GAC TCA GGA TAT GAA GTT CAT CAT CAA AAA TTG GTG TTC TTT GCA His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu Val Phe Phe Ala 605 610 615	1997
GAA GAT GTG GGT TCA AAC AAA GGT GCA ATC ATT GGA CTC ATG GTG GGC Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly Leu Met Val Gly 620 625 630	2045
GGT GTT GTC ATA GCG ACA GTG ATC GTC ATC ACC TTG GTG ATG CTG AAG Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu Val Met Leu Lys 635 640 645	2093
AAG AAA CAG TAC ACA TCC ATT CAT CAT GGT GTG GTG GAG GTT GAC GCC Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val Glu Val Asp Ala 650 655 660 665	2141
GCT GTC ACC CCA GAG GAG CGC CAC CTG TCC AAG ATG CAG CAG AAC GGC Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met Gln Gln Asn Gly 670 675 680	2189
TAC GAA AAT CCA ACC TAC AAG TTC TTT GAG CAG ATG CAG AAC TAGACCCCG Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met Gln Asn 685 690 695	2241
CCACAGCAGC CTCTGAAGTT GGACAGCAAA ACCATTGCTT CACTACCCAT CGGTGTCCAT	2301
TTATAGAATA ATGTGGGAAG AAACAAACCC GTTTATGAT TTACTCATTA TCGCCTTTG	2361
ACAGCTGTGC TGTAAACACAA GTAGATGCCT GAACTTGAAT TAATCCACAC ATCAGTAATG	2421
TATTCTATCT CTCTTTACAT TTTGGTCTCT ATACTACATT ATTAATGGGT TTTGTGTACT	2481

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GTAAAGAATT TAGCTGTATC AAACTAGTGC ATGAATAGAT TCTCTCCTGA TTATTTATCA	2541
CATAGCCCT TAGCCAGTTG TATATTATTC TTGTGGTTG TGACCCAATT AAGTCCTACT	2601
TTACATATGC TTTAAGAATC GATGGGGAT CCTTCATGTG AACGTGGGAG TTCAGCTGCT	2661
TCTCTTGCTT AAGTATTCCCT TTCCCTGATCA CTATGCATTT TAAAGTTAAA CATTAAAG	2721
TATTCAGAT GCTTTAGAGA GATTTTTTT CCATGACTGC ATTTTACTGT ACAGATTGCT	2781
GCTTCTGCTA TATTGTGAT ATAGGAATTA AGAGGATACA CACGTTGTT TCTTCGTGCC	2841
TGTTTTATGT GCACACATTA GGCATTGAGA CTTCAAGCTT TTCTTTTTT GTCCACGTAT	2901
CTTTGGGTCT TTGATAAAGA AAAGAATCCC TGTCATTGT AAGCACTTTT ACGGGGCGGG	2961
TGGGGAGGGG TGCTCTGCTG GTCTCAATT ACCAAGAATT CTCCAAAACA ATTTTCTGCA	3021
GGATGATTGT ACAGAATCAT TGCTTATGAC ATGATCGCTT TCTACACTGT ATTACATAAA	3081
TAAATTAAT AAAATAACCC CGGGCAAGAC TTTCTTTGA AGGATGACTA CAGACATTAA	3141
ATAATCGAAG TAATTTGGG TGGGGAGAAG AGGCAGATT C AATTTCTTT AACCAGTCTG	3201
AAGTTTCATT TATGATACAA AAGAAGATGA AAATGGAAGT GGCAATATAA GGGCATGAGG	3261
AAGGCATGCC TGGACAAACC CTTCTTTAA GATGTGTCTT CAATTTGTAT AAAATGGTGT	3321
TTTCATGTAA ATAAATACAT TCTTGGAGGA GC	3353

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 695 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met	Leu	Pro	Gly	Leu	Ala	Leu	Leu	Leu	Ala	Ala	Trp	Thr	Ala	Arg
1				5				10				15		

Ala	Leu	Glu	Val	Pro	Thr	Asp	Gly	Asn	Ala	Gly	Leu	Leu	Ala	Glu	Pro
				20				25				30			

Gln	Ile	Ala	Met	Phe	Cys	Gly	Arg	Leu	Asn	Met	His	Met	Asn	Val	Gln
			35				40				45				

Asn	Gly	Lys	Trp	Asp	Ser	Asp	Pro	Ser	Gly	Thr	Lys	Thr	Cys	Ile	Asp
			50			55				60					

Thr	Lys	Glu	Gly	Ile	Leu	Gln	Tyr	Cys	Gln	Glu	Val	Tyr	Pro	Glu	Leu
				65		70			75			80			

Gln	Ile	Thr	Asn	Val	Val	Glu	Ala	Asn	Gln	Pro	Val	Thr	Ile	Gln	Asn
				85				90				95			

Trp	Cys	Lys	Arg	Gly	Arg	Lys	Gln	Cys	Lys	Thr	His	Pro	His	Phe	Val
			100			105				110					

Ile	Pro	Tyr	Arg	Cys	Leu	Val	Gly	Glu	Phe	Val	Ser	Asp	Ala	Leu	Leu
				115		120				125					

Val	Pro	Asp	Lys	Cys	Lys	Phe	Leu	His	Gln	Glu	Arg	Met	Asp	Val	Cys
-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----	-----

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130	135	140	
Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu			
145	150	155	160
Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile			
165	170	175	
Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu			
180	185	190	
Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val			
195	200	205	
Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys			
210	215	220	
Val Val Glu Val Ala Glu Glu Glu Val Ala Glu Val Glu Glu Glu			
225	230	235	240
Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu			
245	250	255	
Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile			
260	265	270	
Ala Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Val Arg			
275	280	285	
Val Pro Thr Thr Ala Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu			
290	295	300	
Glu Thr Pro Gly Asp Glu Asn Glu His Ala His Phe Gln Lys Ala Lys			
305	310	315	320
Glu Arg Leu Glu Ala Lys His Arg Glu Arg Met Ser Gln Val Met Arg			
325	330	335	
Glu Trp Glu Glu Ala Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp			
340	345	350	
Lys Lys Ala Val Ile Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu			
355	360	365	
Gln Glu Ala Ala Asn Glu Arg Gln Gln Leu Val Glu Thr His Met Ala			
370	375	380	
Arg Val Glu Ala Met Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn			
385	390	395	400
Tyr Ile Thr Ala Leu Gln Ala Val Pro Pro Arg Pro Arg His Val Phe			
405	410	415	
Asn Met Leu Lys Lys Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His			
420	425	430	
Thr Leu Lys His Phe Glu His Val Arg Met Val Asp Pro Lys Lys Ala			
435	440	445	
Ala Gln Ile Arg Ser Gln Val Met Thr His Leu Arg Val Ile Tyr Glu			
450	455	460	
Arg Met Asn Gln Ser Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala			
465	470	475	480
Glu Glu Ile Gln Asp Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn			
485	490	495	

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Tyr Ser Asp Asp Val Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser
500 505 510
Tyr Gly Asn Asp Ala Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr
515 520 525
Val Glu Leu Leu Pro Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln
530 535 540
Pro Trp His Ser Phe Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn
545 550 555 560
Glu Val Glu Pro Val Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr
565 570 575
Thr Arg Pro Gly Ser Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser
580 585 590
Glu Val Lys Met Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val
595 600 605
His His Gln Lys Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
610 615 620
Gly Ala Ile Ile Gly Leu Met Val Gly Gly Val Val Ile Ala Thr Val
625 630 635 640
Ile Val Ile Thr Leu Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile
645 650 655
His His Gly Val Val Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg
660 665 670
His Leu Ser Lys Met Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys
675 680 685
Phe Phe Glu Gln Met Gln Asn
690 695

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 11
(D) OTHER INFORMATION: /note= ""Xaa at Position 11 is
either Glu or Gln.""

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 27
(D) OTHER INFORMATION: /note= ""Xaa at position 27 is
either Ser or Asn.""

(ix) FEATURE:
(A) NAME/KEY: Modified-site
(B) LOCATION: 28
(D) OTHER INFORMATION: /note= ""Xaa at position 28 is
either Ala or Lys.""

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Xaa Val His His Gln Lys
1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Xaa Xaa
20 25

What Is Claimed Is:

1. An amyloid binding composition for *in vivo* imaging of amyloid deposits comprising:

(a) a labeled amyloid protein or variant thereof that binds to amyloid deposits *in vivo*; and
(b) a pharmaceutically acceptable carrier.

2. The composition of claim 1, wherein said amyloid protein is β -amyloid polypeptide or a variant thereof.

3. The composition of claim 2, wherein said β -amyloid polypeptide variant has the following amino acid sequence (SEQ ID NO:13):

N-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-X-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Y1-Y2-COOH;

wherein X is either Glu or Gln; Y1 is either Ser or Asn; and Y2 is either Ala or Lys.

4. The composition of claim 3, wherein said β -amyloid polypeptide variant is selected from the group consisting of (1) a variant wherein when X is Glu, Y1 is Ser and Y2 is Ala, (2) a variant wherein when X is Glu, Y1 is Asn and Y2 is Lys, and (3) a variant wherein when X is Gln, Y1 is Asn and Y2 is Lys.

5. The composition of claim 2, wherein said β -amyloid polypeptide or variant thereof has an amino acid sequence selected from the following group of amino acid sequences:

(A) (SEQ ID NO:2) Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly-Gly-Val-Val-Ile-Ala-Thr;

(B) (SEQ ID NO:3) Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-

(C) (SEQ ID NO:4) Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Ile-
Val-Ile-Thr-Leu-Val-Met-Leu;
Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-
Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val-
Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-
Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-
Gly-Gly-Val-Val-Ile-Ala-Thr-Val-Ile-
Val-Ile-Thr-Leu-Val-Met-Leu-Lys-Lys-
Lys-Gln-Tyr-Thr-Ser-Ile-His-His-Gly-
Val-Val-Glu-Val-Asp-Ala-Ala-Val-Thr-
Pro-Glu-Glu-Arg-His-Leu-Ser-Lys-Met-
Gln-Gln-Asn-Gly-Tyr-Glu-Asn-Pro-Thr-
Tyr-Lys-Phe-Phe-Glu-Gln-Met-Gln-Asn;
(D) (SEQ ID NO:5) Lys-Thr-Glu-Glu-Ile-Ser-Glu-Val-Lys-
Met-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-
Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-
Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-
Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-
Val-Gly-Gly-Val-Val-Ile-Ala-Thr;
(E) (SEQ ID NO:6) X-Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-
Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-
Val-Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-
Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-
Val-Gly-Gly-Val-Val-Ile-Ala-Thr-Y,

wherein X and Y are one or more APP amino acids which are
not adjacent to β /A4 in the nature;

and

(F) any fragment of (A)-(E), wherein said fragment is
large enough to bind amyloid deposit *in vivo*.

6. The composition of claim 1, wherein said labeled
amyloid protein is radiolabeled amyloid protein.

7. The composition of claim 1, wherein said
radiolabeled amyloid protein is Technetium 99m-labeled
amyloid protein.

8. An *in vivo* method for detecting amyloid deposits
in a subject comprising the steps of

(a) administering to a subject a detectable quantity of an amyloid binding composition comprising a labeled amyloid protein or variant thereof and a pharmaceutically acceptable carrier; and

(b) detecting the binding of the labeled protein or variant thereof to the amyloid deposit.

9. The method of claim 8, wherein said amyloid protein is the β -amyloid polypeptide or variant thereof.

10. The method of claim 8, wherein said amyloid protein is radiolabeled.

11. The method of claim 10, wherein said detecting involves radioactive imaging.

12. The method of claim 8, wherein said administering is selected from the group consisting of intravenous injection, intraventricular injection and a combination of both intravenous and intraventricular injection.

13. The method of claim 8, wherein said amyloid deposits are located in the brain of a subject.

14. A method of diagnosing an amyloidosis-associated disease by detecting amyloid deposits in a subject suspected of having amyloid deposits, said method comprising the steps of:

(a) administering to a subject a detectable quantity of an amyloid binding composition comprising a labeled amyloid protein or variant thereof and a pharmaceutically acceptable carrier; and

(b) detecting the binding of said labeled protein to said amyloid deposit.

15. The method of claim 14, wherein said amyloidosis-associated disease is selected from the group consisting of Alzheimer's Disease and Down Syndrome.

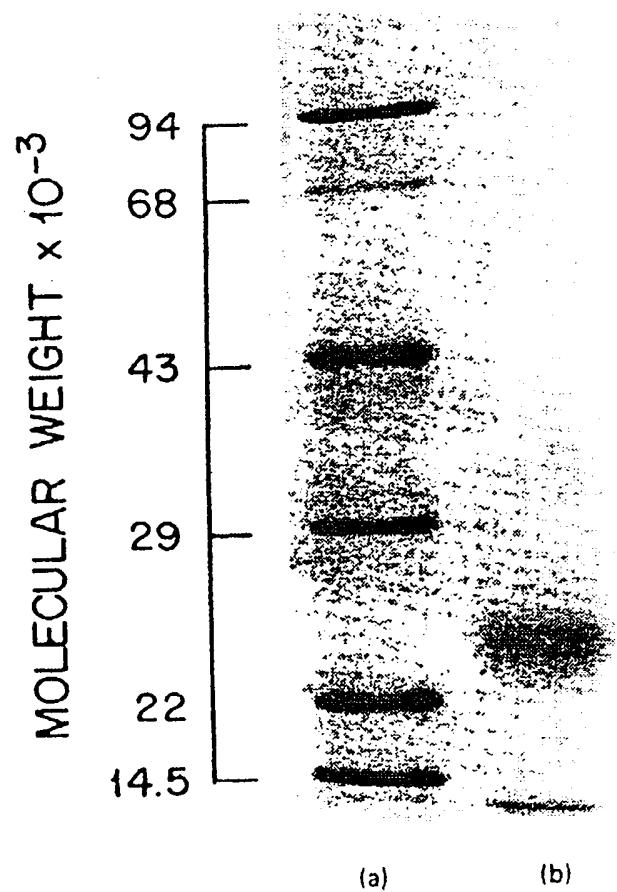
FIG. 1

Clinical Association	Notation	Protein Type
ACQUIRED SYSTEMIC AMYLOIDOSIS		
Immunoglobulin light-chain (primary),	AL	Light chain, type subtype
Multiple myeloma		
Reactive (secondary)	AA	Protein A
Hemodialysis amyloidosis	AH	β_2 microglobulin
HEREDOFAMILIAL		
Polyneuropathy	AF	Prealbumin, variant
Familial Mediterranean fever	AA	Protein A
ORGAN-LIMITED		
Hereditary Icelandic Congophilic angiopathy	ACv _C	Cystatin C, variant
Alzheimer's disease: vessels and plaques	ACv _{B1}	β protein
Senile cardiac	ACp _B	
LOCALIZED ENDOCRINE		
Pancreatic islet	AE _f	Islet amyloid protein [IAP]
Medullary thyroid carcinoma	AE _t	Precalcitonin

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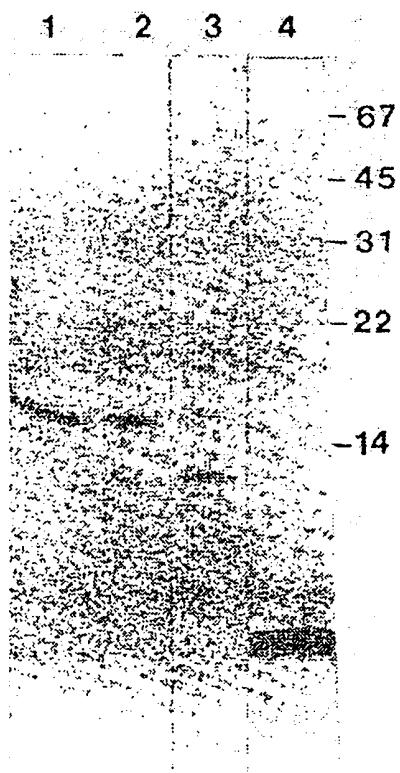
2/10

FIG. 2



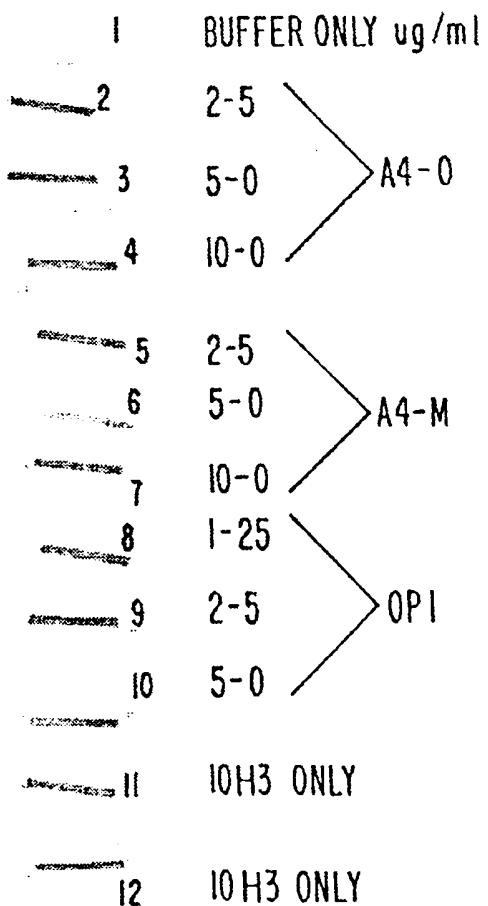
3/10

FIG. 3



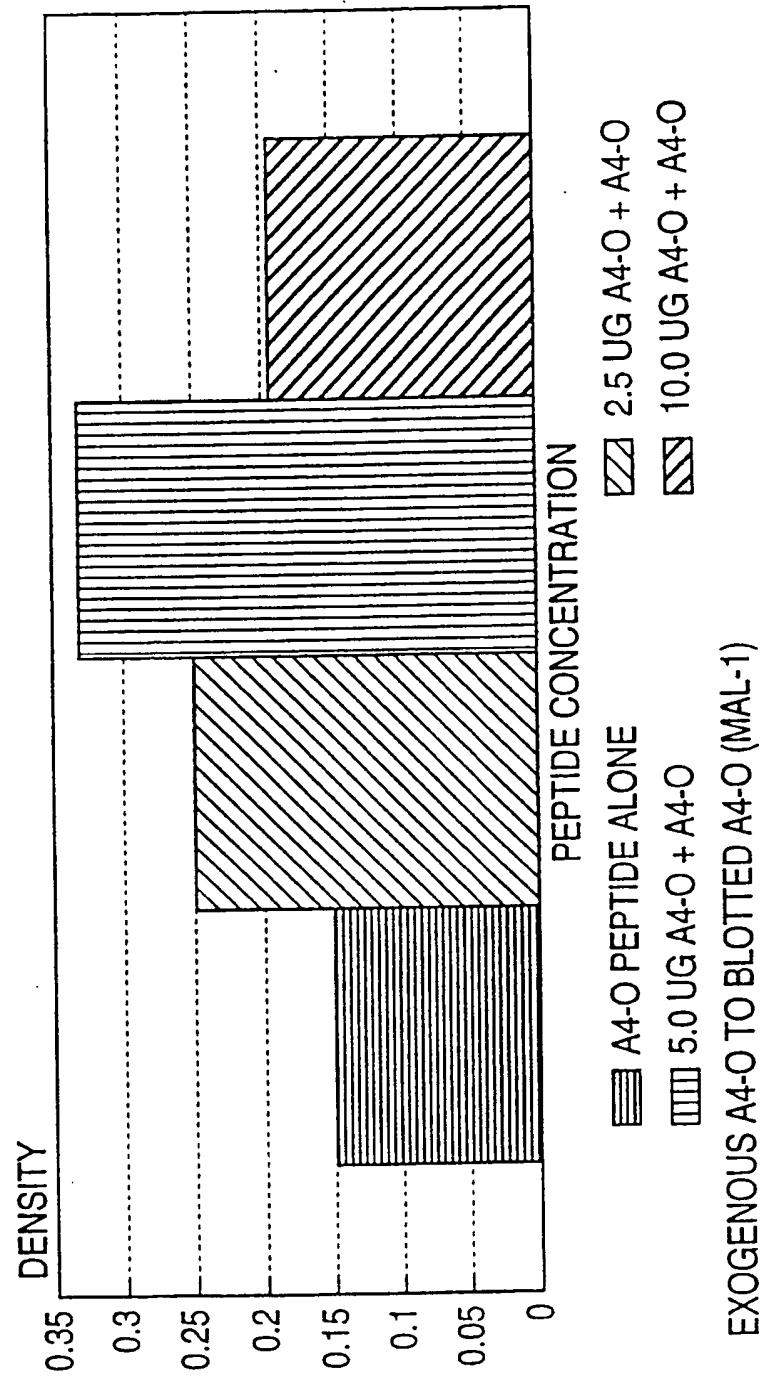
4/10

FIG. 4



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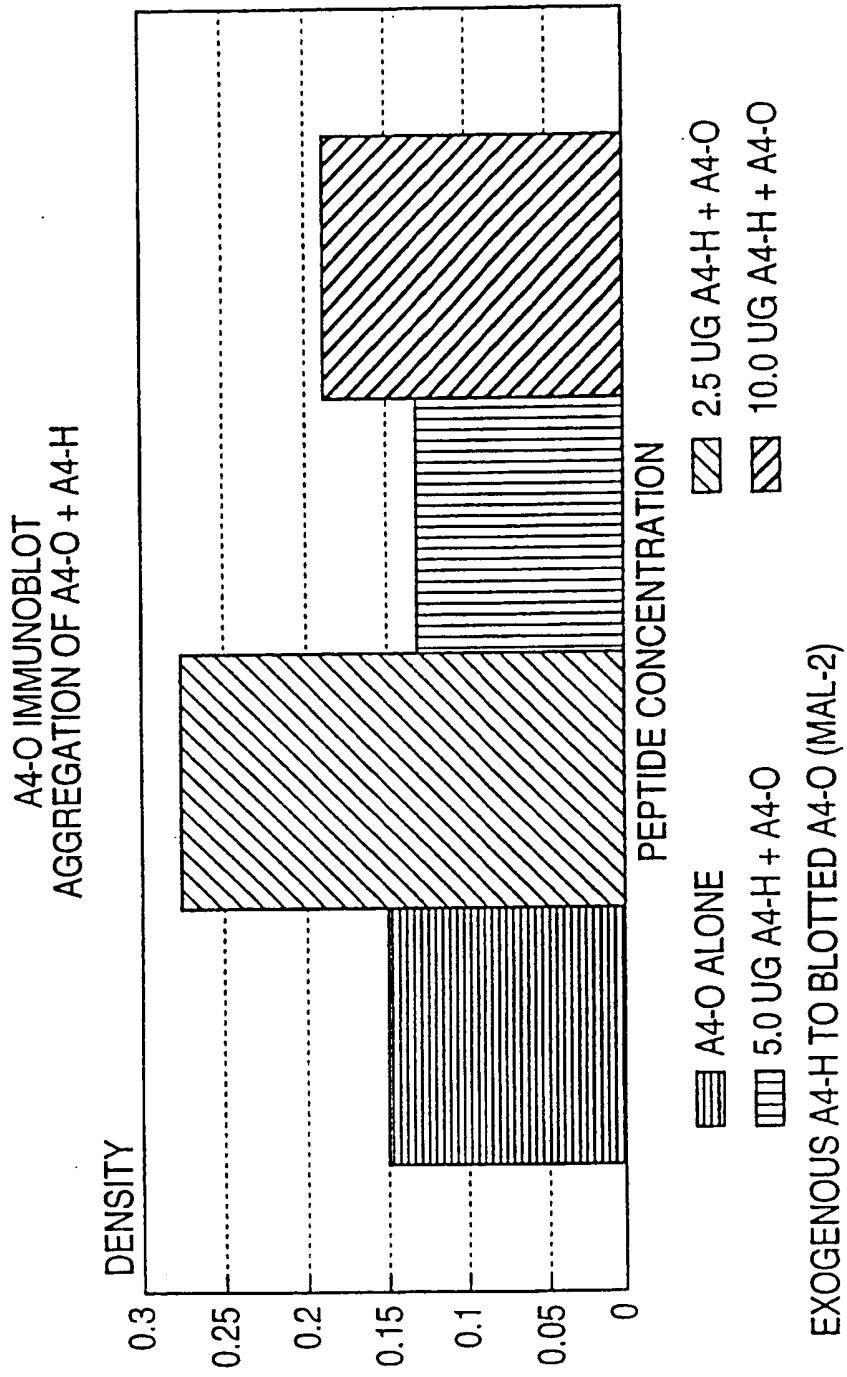
FIG. 5
A4-O IMMUNOBLOT
AGGREGATION OF A4-O + A4-O



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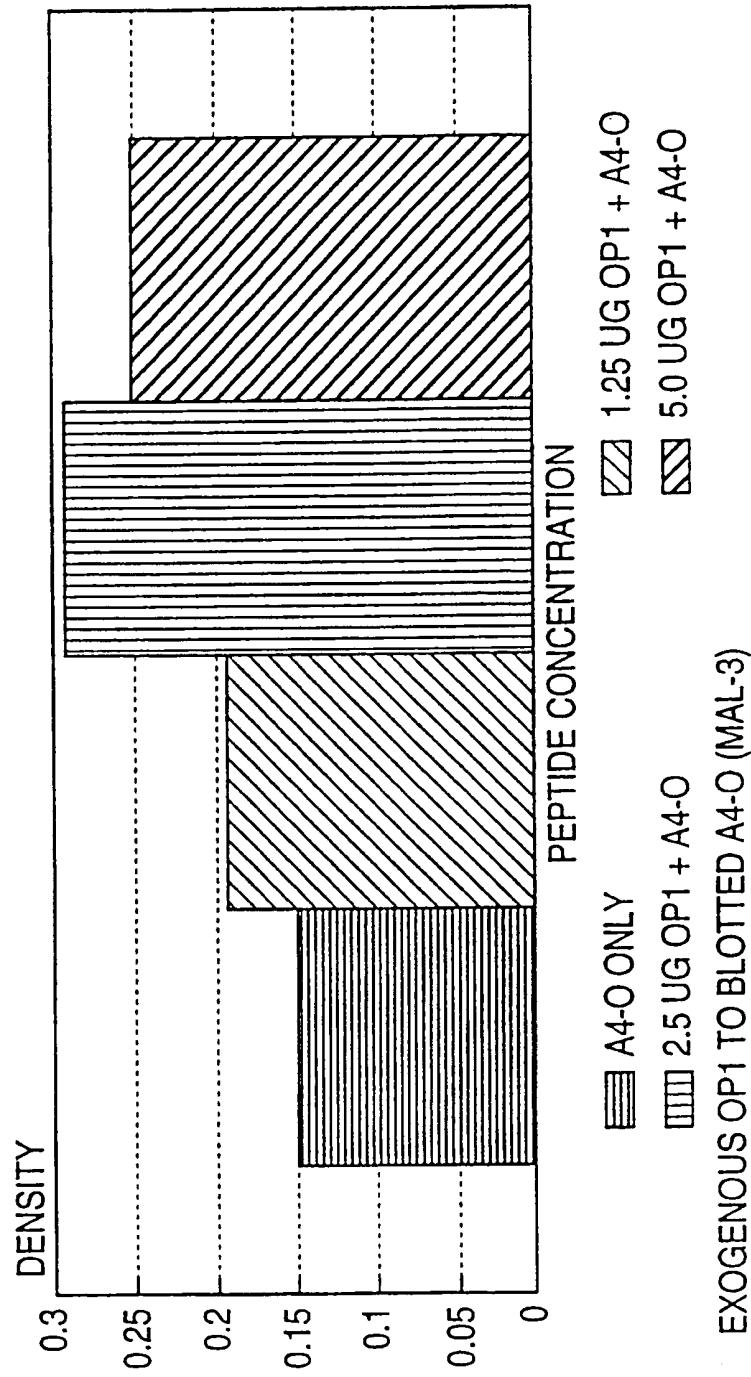
FIG. 6



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FIG. 7
A4-O IMMUNOBLOT
AGGREGATION OF A4-O + OP1



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FIG. 8

10H3 ANTIBODY
APPLIED TO EITHER THE
A4 ANTIGEN OR THE OPI
ANTIGEN.

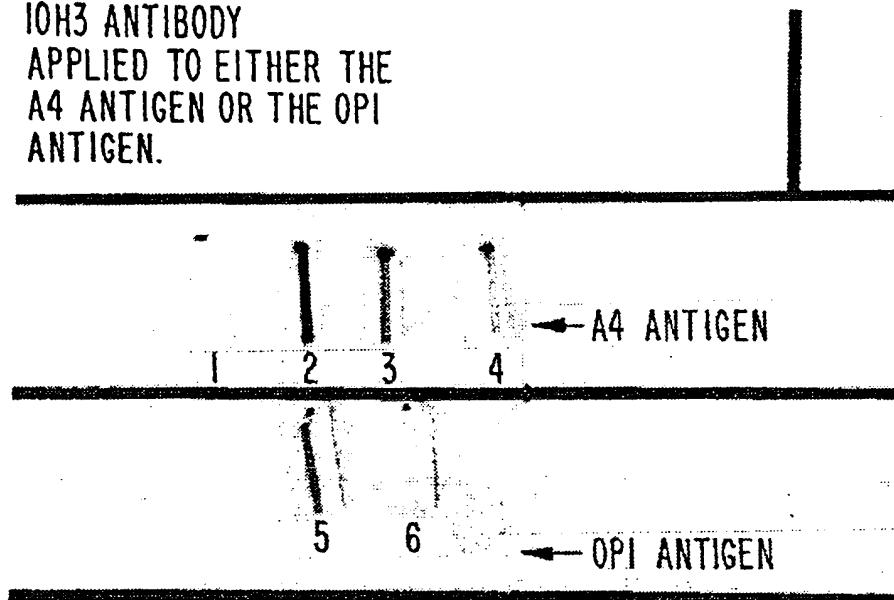


FIG. 9A

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AGTTTCCCTCGCAGCGGTAGCGAGA -121
 2 GCACGCGGAGGAGCGTGCGCGGGCCCCGGAGACCGCGGCGGTGGCGCGCGGGCAGAG -61
 3 CAAGGACGCGGCGGATCCCACTCGCACAGCAGCGCACTCGGTGCCCGCGCAGGGTCGCG -1
 4 ATGCTGCCCGTTGGCACTGCTCCCTGCTGGCCGCTGGACGGCTCGGGCGCTGGAGGTA 60
 M L P G L A L L L A A W T A R A L E V
 1 10 20
 5 CCCACTGATGGTAATGCTGGCCTGCTGGCTGAACCCAGATTGCCATGTTCTGGCAGA 120
 P T D G N A G L L A E P Q I A M F C G R
 30 40
 6 CTGAACATGCACATGAATGTCCAGAATGGGAAGTGGGATTCAAGATCCATCAGGGACAAA 180
 L N M H M N V Q N G K W D S D P S G T K
 50 60
 7 ACCTGCATTGATACCAAGGAAGGCATCCTGCAGTATTCCAAGAAGTCTACCCCTGAAC TG 240
 T C I D T K E G I L Q Y C Q E V Y P E L
 70 80
 8 CAGATCACCAATGTGGTAGAAGCCAACCAACCAGTGACCATCCAGAACTGGTGCAAGCGG 300
 Q I T N V V E A N O P V T I Q N W C K R
 90 100
 9 GCGCGCAAGCAGTGCAAGACCCATCCCCACTTGTGATTCCCTACCGCTGCTTAGTTGGT 360
 G R K Q C K T H P H F V I P Y R C L V G
 110 120
 10 GAGTTTGTAAGTGTGATGCCCTCTCGTTCTGACAAGTCAAATTCTTACACCAGGAGAGG 420
 E F V S D A L L V P D K C K F L H Q E R
 130 140
 11 ATGGATGTTGCGAAACTCATCTTCACTGGCACACCGTCGCCAAAGAGACATGCAGTGAG 480
 M D V C E T H L H W H T V A K E T C S E
 150 160
 12 AAGAGTACCAACTTGCATGACTACGGCATGTTGCTGCCCTGCGGAATTGACAAGTCCGA 540
 K S T N L H D Y G M L L P C G I D K F R
 170 180
 13 GGGGTAGAGTTGTGTTGCCACTGGCTGAAGAAAGTGACAATGTGGATTCTGCTGAT 600
 G V E F V C C P L A E E S D N V D S A D
 190 200
 14 GCGGAGGAGGATGACTCGGATGTCGGTGCGGGCGGACAGACACAGACTATGCAGATGGG 660
 A E E D D S D V W W G G A D T D Y A D G
 210 220
 15 AGTGAAGACAAAGTAGTAGAAGTAGCAGAGGGAGGAAGAAGTGGCTGAGGTGAAAGAA 720
 S E D K V V E V A E E E E V A E V E E E
 230 240
 16 GAAGCCGATGATGACCGAGGACATGAGGATGGTGATGAGGTAGAGGAAGAGGCTGAGGAA 780
 E A D D D E D D E D G D E V E E E A E E
 250 260
 17 CCCTACGAAGAACCCACAGAGAGAACCAACCAGCATGCCACCACCAACCACCAACA 840
 P Y E E A T E R T T S I A T T T T T T T
 270 280
 18 GAGTCTGTGAAAGAGGTGGTCGAGTCCCTACAACAGCAGCCAGTACCCCTGATGCCGTT 900
 E S V E E V V R V P T T A A S T P D A V
 290 300
 19 GACAAGTATCTCGAGACACCTGGGATGAGAATGAAACATGCCATTCCAGAAAGCCAA 960
 D K Y L E T P G D E N E H A H F Q K A K
 310 320
 20 GAGAGGCTTGAGGCCAAGCACCAGAGAGAGAAATGTCCCAGGTATGAGAGAAATGGGAGAG 1020
 E R L E A K H R E R M S Q V M R E W E E
 330 340
 21 GCAGAACGTCAAGCAAAGAACTTGCCTAAAGCTGATAAGAAGGCAGTTATCCAGCATTT 1080
 A E R Q A K N L P A K A D K K A V I Q H F
 350 360
 22 CAGGAGAAAGTGGAAATCTTGGAACAGGAAGCAGCCAAACGAGAGAGACAGCAGCTGGTGGAG 1140
 Q E K V E S L E Q E A A N E R Q Q L V E
 370 380
 23 ACACACATGGCCAGAGTGGAAAGCCATGCTCAATGACCGCCGCGCCTGGCCCTGGAGAAC 1200
 T H M A R V E A M L N D R R R L A L E N
 390 400
 24 TACATCACCGCTCTGCAGGCTGTTCCCTCGGCCCTCGTCACGTGTTCAATATGCTAAAG 1260
 Y I T A L Q A V P R P R H V F N M L K
 410 420
 25 AACTATGTCGCCGAGAACAGAAGGGACAGACAGCACACCCCTAAAGCATTCGAGCATGTG 1320
 C Y V R A E Q K D R Q H T L K H F E H V
 430 440

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FIG. 9B

26 CGCATGGTGGATCCCAAGAAAGCCGTCAGATCCGGTCCCAGTTATGACACACCTCCGT 1380
 R M V D P K K A A Q I R S Q V M T M L R
 450 460

27 GTGATTTATGAGCCATGAATCAGTCTCTCCCTGCTCTACAACTGCCTGCAGTGGCC 1440
 V I Y E R M N Q S L S L L Y N V P A V A
 470 480

28 GAGGAGATTCAGGATGAAGTTGATGAGCTGCTTCAGAAAGAGCAAAACTATTAGATGAC 1500
 E E I Q D E V D E L L Q K E Q N Y S D D
 490 500

29 GTCTGGCCAACATGATTAGTGAACCAAGGATCAGTTACGGAAACGATGCTCTCATGCCA 1560
 V L A N M I S E P R I S Y G N D A L M P
 510 520

30 TCTTGACCGAAACGAAAACCACCGTGGAGCTCCTTCCCGTGAATGGAGAGTCAGCCTG 1620
 S L T E T K T T V E L L P V N G E F S L
 530 540

31 GACGATCTCCAGCCGGCATTTGGGCTGACTCTGTGCCAGCCAACACAGAAAAC 1680
 D D L Q P W H S F G A D S V P A N T E N
 550 560

32 GAAGTTGAGCCTGTTGATGCCGCCCTGCTGCCGACCGAGGACTGACCACTCGACCAGGT 1740
 E V E P V D A R P A A D R G L T T R P G
 570 580

33 TCTGGTTGACAAATATCAAGACGGAGGAGATCTGAAGTGAAGATGGATGCAGAATTC 1800
 S G L T N I K T E E I S E V K M D A E F
 590 600

34 CGACATGACTCAGGATATGAAGTTCATCATCAAAATTGGTGTCTTGAGAAGATGTG 1860
 R H D S G Y E V H H O K L V F F A E D V
 610 620

35 GGTTCAAACAAAGGTGCAATCATTGGACTCATGGTGGCGGTGTTGTCATAGCGACAGTG 1920
 G S N K G A I I G L M V G G V V I A T V
 630 640

36 ATCGTCATCACCTTGGTGTGAAGAAGAAACAGTACACATCCATTATCATGGTGTG 1980
 I V I T L V M L K K Q Y T S I H H G V
 650 660

37 GTGGAGGTTGACGCCGCTGTCACCCCAGAGGGAGCGCCACCTGTCCAAGATGCAGCAGAAC 2040
 V E V D A A V T P E E R H L S K M Q Q N
 670 680

38 GGCTACGAAAATCCAACCTACAAGTTCTTGAGCAGATGCAGAACTAGACCCCCGCCACA 2100
 G Y E N P T Y K F F E Q M Q N *
 690

39 GCAGCCTCTGAAGTTGGACAGCAAAACCATTGCTTCACTACCCATCGGTGTCATTATA 2160

40 GAATAATGTGGAGAAACAAACCGTTTATGATTACTCATATCGCCTTTGACAGC 2220

41 TGTGCTGTAACACAAGTAGATGCCTGAACCTGAATTAAATCCACACATCAGTAATGTATTTC 2280

42 TATCTCTTTACATTGGTCTCTATACTACATTATAATGGTTTGTACTGTAAA 2340

43 GAATTAGCTGTATCAAACACTAGTGCATGAATAGATTCTCTCTGATTATTATCACATAG 2400

44 CCCCTTAGCCAGTTGTATATTATTCTTGTTGTGACCCAATTAGCTTACTTACA 2460

45 TATGTTAAGAATCGATGGGGATGCTCATGTGAAACGTGGAGTTGAGCTGCTTCTCT 2520

46 TGCCTAAGTATTCCCTTCCTGATCACTATGCATTAAAGTAAACATTAAAGTATT 2580

47 CAGATGTTAGAGAGATTTTTCCATGACTGCATTACTGTACAGATTGCTGCTTC 2640

48 TGCTATATTGTGATATAGGAATTAAAGAGGATACACACGTTGTTCTCGTGCCTGTT 2700

49 TATGTGCACACATTAGGCATTGAGACTTCAAGCTTTCTTTGTCCACGTATCTTG 2760

50 GGTCTTGATAAAAGAAAAGAACCTCTGTCATTGTAAGCAGCTTACGGGGCGGGTGGGG 2820

51 AGGGGTGCTCTGCTGGTCTCAATTACCAAGAATTCTCCAAAACAATTCTGCAGGATG 2880

52 ATTGTACAGAATCATTGCTTATGACATGATCGTTCTACACTGTATTACATAAATAAT 2940

53 TAATAATAACCCCGGGCAAGACTTTCTTGAGGATGACTACAGACATTAAATAAT 3000

54 CGAAGTAATTGGTGGGGAGAAGAGGGAGATTCAATTCTTAACCAGTCTGAAGTT 3060

55 TCATTTATGATACAAAAGAAGATGAAAATGGAAGTGGCAATATAAGGGATGAGGAAGGC 3120

56 ATGCCTGGACAAACCTTCTTTAAGATGTGCTTCATTTGTATAAAATGGTGTTC 3180

57 TGTAAATAATACATTCTGGAGGAGC-poly(A) tall

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/05809

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :G01N 33/367; A61K 49/00, 43/00

US CL :435/7.21; 424/1.11, 1.57, 9; 514/2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/7.1, 7.2, 7.21; 424/1.11, 1.57, 1.69, 9; 514/2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Medline, EMBASE, BIOSIS, CA Search, WPO, APS, IntelliGenetics

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A, 93/04194 (MAGGIO ET AL) 04 March 1993, see entire document, especially page 4, lines 8-15, page 8, lines 14-17, page 12, lines 15-39 and page 14, lines 26-36.	1, 2, 6, 7, 8-15
Y	Proceedings of the National Academy of Science USA, Volume 87, issued June 1992, Maggio et al, "Reversible in vitro growth of Alzheimer disease beta-amyloid plaques by deposition of labeled amyloid peptide", pages 5462-5466, especially see Abstract on page 5462.	3-5
Y		1-15

Further documents are listed in the continuation of Box C. See patent family annex.

• Special categories of cited documents:		
"A"	document defining the general state of the art which is not considered to be of particular relevance	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"E"	earlier document published on or after the international filing date	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

24 AUGUST 1994

Date of mailing of the international search report

SEP 06 1994

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Society for Neuroscience Abstracts from the 21st Annual Meeting, Volume 17, Number 1-2, issued Nov. 1991, Mantyh et al, "Distribution and Characterization of Amyloid Beta Protein Deposition in Normal Human and Alzheimer's Diseased Cerebral Cortex Using ¹²⁵ I-BAP as the Radioligand", page 912, Abstract 364.4, see entire abstract.	1, 2, 6, 7 3-5, 8-15
Y	Nature, Volume 325, issued 19 February 1987, Kang et al "The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor", pages 733-735, especially see Fig. 2.	1-15
A	Freeman et al, editors, Physician Desk Reference For Radiology and Nuclear Medicine, published 1977 by Medical Economics Company, Oradell, N.J., pages 39-43.	1-15
A	Proceedings of the National Academy of Science USA, Volume 88, issued August 1991, Kowall et al, "An in vivo model for the neurodegenerative effects of beta amyloid and protection by substance P", pages 7247-7251.	1-15
A	Neurobiology of Aging, Volume 13, Number 5, issued 1992, May et al, "Beta-Amyloid Peptide In Vitro Toxicity: Lot-to-Lot Variability", pages 605-607.	1-15